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(71) Applicant: **COLORADO SCHOOL OF MINES**  
[US/US]; 1500 Illinois Street, Golden, CO 80401 (US).

(72) Inventors: **Oakey, John**; 1006 13th Street, Golden, CO  
80401 (US). **MARR, David, W.M.**; 21508 Mountsfield  
Drive, Golden, CO 80403 (US).

(74) Agent: **KULISH, Christopher, J.**; Holland & Hart LLP,  
P.O. Box 8749, Denver, CO 80201-8749 (US).

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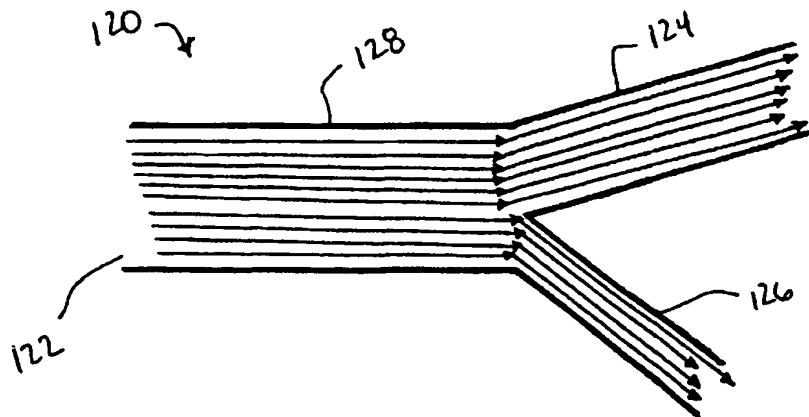
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(54) Title: LAMINAR FLOW-BASED SEPARATIONS OF COLLOIDAL AND CELLULAR PARTICLES



(57) Abstract: A system, method and apparatus employing the laminar nature of fluid flows in microfluidic flow devices in separating, sorting or filtering colloidal and/or cellular particles from a suspension in a microfluidic flow device is disclosed. The microfluidic flow devices provide for separating a particle in a suspension flow in a microfluidic flow chamber (120). The chamber (120) includes a microfluidic flow channel (128) comprising at least one inlet port (122) for receiving a suspension flow under laminar conditions, a first outlet port (124) and a second outlet port (126). The chamber (120) further includes an interface for translating a particle

within the channel. The first outlet port (124) receives a first portion of the suspension exiting the channel (128) and the second outlet (126) port receives the particle in a second portion of the suspension exiting the channel (128).

WO 03/066191 A1

## LAMINAR FLOW-BASED SEPARATIONS OF COLLOIDAL AND CELLULAR PARTICLES

### RELATED APPLICATIONS

5           This application claims the priority benefit of United States Provisional Patent Application Serial No. 60/354,372 filed on 4 February 2002 is herein incorporated in its entirety.

### FIELD OF THE INVENTION

10           The present invention relates to a general class of devices that uniquely employ laminar flows in separating, filtering or sorting colloidal or cellular particles from a suspension within microfluidic devices.

### BACKGROUND OF THE INVENTION

15           Microfluidic flows are particularly useful due to their ultra laminar nature that allows for highly precise spatial control over fluids, and provides both unique transport properties and the capability for parallelization and high throughput. These qualities have made microfluidic platforms a successful option for applications in printing, surface patterning, genetic analysis, molecular separations and sensors. Specifically, the effective  
20           separation and manipulation of colloidal and cellular suspensions on the microscale has been pursued with keen interest due to the tremendous multidisciplinary potential associated with the ability to study the behavior of individual particles and cells. Devices that employ electric fields to direct flow for the purpose of sorting and manipulating populations of cells have been realized and in some cases have demonstrated potential to  
25           achieve efficiencies comparable to their conventional analog, fluorescent activated cell sorters (FACS).

### SUMMARY OF THE INVENTION

30           The present invention relates to a system, method and apparatus employing the laminar nature of fluid flows in microfluidic flow devices in separating, sorting or filtering colloidal and/or cellular particles from a suspension in a microfluidic flow device. In one embodiment, a microfluidic flow device is provided for separating a particle within a suspension flow in a microfluidic flow chamber. The chamber includes

-2-

a microfluidic channel comprising an inlet port for receiving a suspension flow under laminar conditions, a first outlet port and a second outlet port. The chamber further includes an interface for translating a particle within the channel. The first outlet port receives a first portion of the suspension exiting the channel and the second outlet port receives the particle in a second portion of the suspension exiting the channel.

5 An alternative microfluidic flow device for separating a particle from a suspension flow into a second fluid flow is also provided. The microfluidic flow device includes a microfluidic channel comprising a first inlet port for receiving the suspension flow, a second inlet port for receiving the second fluid flow, a first outlet port and a second outlet  
10 port. The channel is adapted to receive the suspension flow and the second fluid flow under laminar conditions. The device further includes an interface for translating a particle from the suspension flow to the second fluid flow. The first outlet port is adapted to receive at least a portion of the suspension flow exiting the channel and the second outlet port is adapted to receive the particle in at least a portion of the second fluid flow  
15 exiting channel.

A method of separating a particle within a suspension is also provided in which a suspension flow is received in a microfluidic channel under laminar conditions. A particle in the suspension is translated within the suspension flow. A first portion of the suspension flow exits through a first outlet port, and the particle exits in a second portion  
20 of the suspension flow through a second outlet port.

Another method of separating a particle from a suspension flow is provided in which a suspension flow and a second fluid flow are received in a microfluidic channel. The suspension and the second fluid flow under laminar conditions in the channel. A particle is separated from the suspension flow into the second fluid flow. At least a  
25 portion of the suspension flow exits through a first outlet port, and the particle exits in at least a portion of the second fluid flow through a second outlet port.

A cartridge is also provided for use in system to separate a particle from a suspension flow. The cartridge comprises a microfluidic channel including an inlet port for receiving a suspension flow under laminar conditions, a first outlet port and a second  
30 outlet port. The cartridge further comprises an interconnect for connecting the cartridge to the system. The microfluidic channel is adapted to receive the suspension flow and provide an environment for translating the particle within the suspension flow. The first

-3-

outlet port is adapted to receive a first portion of the suspension flow, and the second outlet port is adapted to receive the particle in a second portion of the suspension flow.

An alternative cartridge is further provided for use in system to separate a particle from a suspension flow into a second fluid flow. The cartridge comprises a microfluidic channel including a first inlet port for receiving the suspension flow, a second inlet port for receiving the second fluid flow, a first outlet port and a second outlet port. The channel is further adapted to receive the suspension flow and the second fluid flow in the channel under laminar conditions. The cartridge further comprises an interconnect for connecting the cartridge to the system. The microfluidic channel is adapted to provide an environment for translating the particle from the suspension flow to the second fluid flow. The first outlet port is adapted to receive at least a portion of the suspension flow, and the second outlet port is adapted to receive the particle in at least a portion of the second fluid flow.

A system for separating a particle from a solution in a microfluidic flow device is also provided. The system includes a detector, an information processor and an actuator. The detector monitors a microfluidic channel of the microfluidic flow device and provides an output to the information processor. The information processor processes the output to determine if the particle is present. If the particle is present, the information processor triggers the actuator to translate the particle within the channel.

A microfluidic chemical dispenser for dispensing a fluid flow into a plurality of receptacles is further provided. The dispenser comprises a first inlet port, a second inlet port, a third inlet port, a central channel, a plurality of outlet ports, and a modulator. The channel is adapted to receive, under laminar conditions, a first fluid flow through the first input port, a second fluid flow through the second input port and a third fluid flow through the third input port. The second input port is positioned at a first angle to the first input port, and the third input port is positioned at a second angle to the first input port. The modulator modulates the flow rates of the second and third fluid flows to dispense the first fluid flow into a plurality of outlet ports.

The foregoing and other features, utilities and advantages of the invention will be apparent from the following more particular description of a preferred embodiment of the invention as illustrated in the accompanying drawings.

-4-

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts a flow diagram of an actuated process of separating a colloidal or cellular particle from a suspension in a microfluidic flow device;

5 Figure 2 depicts a block diagram of an exemplary system for separating a colloidal or cellular particle from a suspension in a microfluidic flow device;

Figure 2a depicts a block diagram of a microfluidic flow network that may be used in conjunction with the system depicted in Figures 2, 3 and 4;

Figure 3 depicts a block diagram of an alternative system for separating a colloidal or cellular particle from a suspension in a microfluidic flow device;

10 Figure 4 depicts a block diagram of another alternative system for separating a colloidal or cellular particle from a suspension in a microfluidic flow device, wherein the system controls a valve actuator to separate the particle from the suspension;

Figure 5 depicts a fluid flow path in one example of a microfluidic flow chamber;

15 Figure 5a depicts a particle entering the microfluidic flow chamber depicted in Figure 5 via an inlet port;

Figure 5b depicts the particle depicted in Figure 5a being moved within a central channel of the microfluidic flow chamber depicted in Figure 5;

Figure 5c depicts the particle depicted in Figure 5a exiting the central channel of the microfluidic flow chamber depicted in Figure 5 via an outlet port;

20 Figure 6 depicts side-by-side laminar fluid flows in the central channel of the microfluidic flow chamber depicted in Figure 5;

Figure 6a depicts a particle entering the central channel via an inlet port of the microfluidic flow chamber in the first fluid flow depicted in Figure 6;

25 Figure 6b depicts the particle depicted in Figure 6a being moved within the central channel of the microfluidic flow chamber from the first flow to the second flow;

Figure 6c depicts the particle depicted in Figure 6a exiting the central channel of the microfluidic flow chamber in the second flow via an outlet port;

Figure 7 depicts an alternative example of a microfluidic flow chamber;

30 Figure 7a depicts side flows pinching a central flow of a suspension at the entrance to a central channel of the microfluidic flow chamber depicted in Figure 7 to orient the flow of suspension in the center portion of the channel;

-5-

Figure 7b depicts side flows pinching a central flow of a suspension at the entrance to a central channel of the microfluidic flow chamber depicted in Figure 7 to orient the flow of suspension in the bottom portion of the channel;

Figure 7c depicts side flows pinching a central flow of a suspension at the  
5 entrance to a central channel of the microfluidic flow chamber depicted in Figure 7 to orient the flow of suspension in the top portion of the channel;

Figure 8 depicts another example of a microfluidic flow chamber including a plurality of outlet ports for sorting colloidal and/or cellular particles in a suspension;

Figure 9 depicts a microfluidic flow chamber including a mechanical actuator for  
10 separating a colloidal and/or cellular particle in a suspension, wherein the mechanical actuator comprises a valve;

Figure 9a depicts an alternative example of a microfluidic flow chamber including a mechanical actuator for separating a colloidal and/or cellular particle in a suspension, wherein the mechanical actuator comprises a valve;

Figure 9b depicts the particle being separated from the suspension via the valve of  
15 the microfluidic chamber depicted in Figure 9a being closed to divert the particle into an alternative outlet port;

Figure 9c depicts the particle exiting the alternative outlet port of the microfluidic chamber depicted in Figure 9a and the valve retracting to its open position;

Figure 9d depicts another alternative example of a microfluidic flow chamber  
20 including a chemical actuator for separating a colloidal and/or cellular particle in a suspension, wherein the chemical actuator comprises a chemically actuated valve;

Figure 9e depicts the particle being separated from the suspension via the valve of  
25 the microfluidic chamber depicted in Figure 9d being swollen closed to divert the particle into an alternative outlet port;

Figure 9f depicts the particle exiting the alternative outlet port of the microfluidic chamber depicted in Figure 9d and the valve shrinking to its open position;

Figure 10 depicts a series of suspensions being introduced into a microfluidic flow chamber in series separated by buffers;

Figure 11 depicts an alternative non-actuated microfluidic flow device for  
30 separating colloidal and/or cellular particles from a suspension;

-6-

Figure 12 depicts another alternative non-actuated microfluidic flow device for separating colloidal and/or cellular particles from a suspension;

Figure 13 depicts an exemplary non-actuated microfluidic flow device for sorting colloidal and/or cellular particles from a suspension by size;

5        Figure 14 depicts an alternative non-actuated microfluidic flow device for separating motile cellular particles from a suspension;

Figure 15 depicts an exemplary non-actuated microfluidic flow device for separating colloidal and/or cellular particles from a suspension; and

Figure 16 depicts a cartridge including a microfluidic flow chamber.

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#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The processes and devices described herein relate to actuated or non-actuated separation of various colloidal and/or cellular particles from a suspension flowing under laminar conditions in a microfluidic flow device. The colloidal and cellular particles may  
15        include, for example, polymeric, inorganic or other abiotic colloidal particles, individual polymers, proteins, fragments of DNA or RNA, entire sections or genomes of DNA, cells including single-celled organisms, formed bodies such as they would appear in blood, viruses and the like. A microfluidic flow device, as used for the purposes of the present invention, refers to a microscale device that handles volumes of liquid on the order of  
20        nanoliters or picoliters.

Under "laminar" flow conditions, a fluid flows through a channel without turbulence. The quantification of laminar or nonturbulent behavior is typically done through calculation of the Reynolds number,  $Re = \rho v D / \eta$ , where  $\rho$  is the fluid density,  $\eta$  is the fluid viscosity,  $v$  is the fluid velocity, and  $D$  is some characteristic channel  
25        dimension (typically the channel width). If the Reynolds number is small ( $<1000$ ) for typical channel geometries, then flow is laminar, reversible, and non-turbulent. For this reason, the diameter of the channel can be designed to account for the intended fluid properties and fluid velocity, or, equivalently, the fluid velocity can be determined by the fluid properties and the channel diameter.

30        Figure 1 shows a flow diagram of a process for an actuated separation of colloidal and/or cellular particles from a suspension flowing through a microfluidic flow device under laminar conditions. In the receive input block 10, an input is received from a

-7-

sensor monitoring a target region for a particle of interest. The target region may be monitored to detect any known attribute (or absence thereof) that can be used to distinguish a particle from the remaining suspension. An imaging device such as a charge-coupled device (CCD) camera, for example, may be utilized to capture a stream of images that may be used to identify a particle by its particular morphological attributes or motility. Alternatively, signatures, fingerprints or indices such as a fluorescent signature, light scattering signature, optical fingerprint, X-ray diffraction signature or index of refraction, and the like, or any combination of these, may be used to distinguish the particle from the remaining suspension. Surface charges of particles may also be used to distinguish the particle by observing the reaction of the particle to an applied electric or magnetic field.

Further, the suspension or the individual particles may be pretreated, as known in the art, to enhance the recognition of the particles. The suspension may further be pretreated with an antibody that will bind specifically to a particular type of particle may be used to enable or enhance the recognition of the particle. A suspension of cells, for example, may be pretreated with antibody-decorated magnetic particles and passed through a magnetic field to distinguish the particles from the remaining suspension. Similarly, other recognition methodologies known in the art may be used to distinguish the particle of interest from the remaining suspension.

Information processing block 20 performs any processing steps necessary to distinguish the particle from the remaining suspension such as comparing received images or signals from the receive input block 10 to threshold values, e.g., size and shape. The information processing block 20 may include any required processing steps as known in the art to distinguish the particle of interest from the remaining suspension. The processing steps may vary depending upon the type of input received. The processing step, for example, may include simple recognition of a digital input value or may include complicated processing steps to detect whether a given input corresponds to the presence of a particle of interest.

After a particle is identified, the particle may be separated from the suspension by the actuation of separation block 30. The actuation may include, for example, steering an optical trap such as via a piezoelectric mirror, an acoustic optic deflector, a diffraction grating, a holographically-generated trap, a static line trap, a dynamic line trap, an optical



-8-

gradient, a microlens array, a waveguiding structure or other known optical steering mechanism. The actuation may alternatively include generating an electric field or a magnetic field. The actuation may also include a mechanical or chemical actuator. A mechanical actuator, for example, may include a pump, valve, gate, applied pressure and the like. A chemical actuator, for example, may include a hydrogel or similarly behaving material that reacts to a property sensed in the suspension that may indicate the presence or absence of a particle of interest.

Each of the functions shown in blocks 10, 20 and 30 of Figure 1, however, need not be performed by distinct hardware components. A sensor, for example, may receive an input and perform the information processing on that input to determine if a particle of interest has been detected. An actuator may even perform each of the functions by directly reacting to a property being monitored (e.g., a pH responsive hydrogel may swell in response to a sensed pH level).

Figure 2 shows one exemplary system 40 for separating a particle of interest from a suspension in a microfluidic flow device 44 utilizing an actuated separation technique. The system includes an detector system 50, an information processing system 60 and an actuator system 70. The detector system 50 includes an imaging system, such as a camera 52, that may be used to image a field of view through a filter 54 and a microscope 56. The detector system 50, for example, may utilize a CCD camera to capture a stream of images of the microfluidic flow device through a microscope lens. In one particular embodiment, the camera 52 captures images at a rate of 30 images per second through a 100X objective. The images are recorded by a recording device, such as VCR 58, and/or passed directly to an information processor, such as a computer 62. Optionally, the identification of the particles may be aided by utilizing the laser 74 or another light source, such as a secondary laser, multiple other lasers, a broad spectrum lamp and the like, to irradiate the suspension to illuminate the particles of interest.

The information processor may include the computer 62, a controller or other processor known in the art. The information processor receives and processes the image data and distinguishes the particle of interest from the remaining suspension as described above. Once the particle is recognized, the information processor may trigger the actuator system 70 to separate the particle from the suspension.

The actuator system 70 may include a targeting device 72 to target a laser beam from a laser 74 on the microfluidic flow device 44. The targeting device, for example, may include a piezo drive 76 to control a piezo mirror 78 to direct the beam of a laser 74. The laser 74, when focused on the particle, traps the particle. The optical trap may then  
5 be used to translate the particle between streams in the channel of the microfluidic flow device 44.

Utilizing an optical trap as the means of actuation provides the capability for highly precise and immediately customizable individual separations. Other applied fields, however, may also be utilized to translate particles from the primary stream to the  
10 secondary stream. Both electric and magnetic fields may be employed with appropriate suspensions to isolate individual or multiple particles. All colloidal particles and living cells carry with them a surface charge, which, in the presence of an electrical field results in electrophoresis. The electrophoretic force, or the migration of surface ions with an electric field, is sufficient to translate cells or particles from one stream to another.  
15 Similarly, if a particle or cell possesses a magnetic moment, it may be selectively translated in a magnetic field. Each of these fields could be applied continuously to fractionate particles or cells based on electrical or magnetic properties, or could be pulsed or applied discriminatively for custom separations.

As described above, the suspension or the individual particles may be pretreated,  
20 as known in the art. The pretreatment, for example, may enhance the response of the particle to an optical trap or electric or magnetic field. The suspension may further be pretreated with items, such as antibodies that will bind specifically to a particular type of particle may be used to enable or enhance the movement of the particle via an optical trap or electric or magnetic field. A suspension of cells, for example, may be pretreated with  
25 antibody-decorated magnetic particles and, thus, be easily moved by means of a magnetic field.

Figure 2a shows further detail of a microfluidic flow device 44 that may be used in connection with a system 40, 80 and 110 such as shown in Figures 2, 3 and 4, respectively. The microfluidic flow device 44 includes a flow generator 45, which  
30 provides a pressure differential to induce fluid flows through the microfluidic flow device 44. The pressure differential, for example, may be induced by any method known in the art such as, but not limited to, capillary forces; gravity feed; electro-osmosis systems;

-10-

syringes; pumps such as syringe pumps (e.g., a kdScientific, model 200 syringe pump), peristaltic pumps and micropumps; valves such as three-way valves, two-way valves, ball valves and microvalves; suction; vacuums and the like. Further, although Figure 2a shows the flow generator located upstream of a microfluidic flow chamber 47, the flow generator may also be placed midstream in the microfluidic flow chamber 47 or downstream of the microfluidic flow chamber 47. Further, the microfluidic flow chamber 47 preferably provides at least one output 49 with the collected particles separated from a suspension within the chamber. This output 49 may provide the collected particles as an end process or may provide the particles to a downstream network for further processing.

Figure 3 shows an alternative system for separating a particle of interest from a suspension in a microfluidic flow device. The imaging system 90 and its operation is the same as shown in Figure 2 except that the imaging system 90 further includes a field generator 92. The field generator 92 induces an electric or magnetic field in the microfluidic flow device 44. As the suspension flows through the device 44, the movement of the particles of interest, whether induced by electric or magnetic properties of the particles themselves or by properties associated with a pretreatment of the particles, is captured by the imaging system 90 and identified by the information processor 100.

Figure 4 shows another system 110 for separating a particle of interest from a suspension in a microfluidic flow device 114. In this system, the actuator system includes a valve controller 112 that controls the operation of a valve within the microfluidic flow device 114. The valve, for example, may be opened to divert the flow of the suspension within the microfluidic flow device for a predetermined time after the recognition of the particle of interest. In this manner, the system separates the particle in a small portion of the suspension by diverting the suspension carrying the particle into an alternative outlet port. An example of such a valve is described below with respect to Figures 9a – 9c.

A particular microfluidic flow channel can be modeled to determine the flow path of a fluid flowing in a laminar manner through the channel. This is well known in the art and involves solving the Langevin equations, the Navier-Stokes equations or other equations of motion, which can be done manually or electronically. Commercial software tools are also available for modeling the laminar flow path of a fluid through any microfluidic flow channel. For example, CFDASE, a finite element modeling for computational fluid dynamics module available from Open Channel Foundation

Publishing Software from Academic & Research Institutions of Chicago, Illinois, and FIDAP, a flow-modeling tool available from Fluent, Inc. of Lebanon, New Hampshire, can be used to model the laminar flow of a fluid through a particular microfluidic channel.

5           Figure 5 shows an embodiment of a microfluidic flow chamber 120 in which a particle of interest may be separated from a suspension. The microfluidic flow chamber includes a single inlet port 122, two outlet ports 124 and 126 and a central channel 128. Figure 5 further shows arrows depicting a modeled laminar flow of a particular fluid through the microfluidic flow chamber 120. Figures 5a-5c show a process for separating  
10           a particle 130 from a suspension flow in the microfluidic flow chamber 120 of Figure 5. Figure 5a shows the particle entering the microfluidic flow chamber 120 via the inlet port 122 at which point it is identified as described above. The information processor initiates an actuator to direct the particle 130 into a desired portion of the flow stream 132 of the suspension in Figure 5b. Thus, the particle 130 is directed to a portion of the flow in  
15           which it will exit the central chamber 128 through the second outlet port 126, as shown in Figure 5c.

          Figures 6 and 6a-6c show an alternative embodiment of a microfluidic flow chamber 140, which includes two inlet ports 142 and 144, a central channel 146 and two outlet ports 148 and 150. As Figure 6 shows, a first fluid 152, indicated by dye, enters the  
20           central channel 146 via the first inlet port 142 and a second fluid 154 enters the central channel 146 via the second inlet port 144. As described above, when the first fluid 152 and the second fluid 154 flow through the microfluidic flow chamber in a laminar manner, the fluids maintain separate streams and undergo minimal convective mixing. Rather, the mixing present is primarily due to molecular-scale diffusion, which for  
25           colloidal-sized particles is referred to as Brownian movement, as shown near the outlet port of the central channel. The system can be designed to minimize the diffusion that occurs within the central channel 146 by controlling the central channel 146 dimensions and the velocity of the fluid flowing through the channel 146. In general, the diffusion distance  $x$ , can be expressed as  $x \approx \sqrt{D \cdot t}$ , wherein  $D$  is the diffusivity and  $t$  is the  
30           time. To a first order, the diffusivity is inversely proportional to the size of the particle. Therefore, to a first order, the channel residence time required to achieve complete mixing,  $t \approx x^2 D^{-1}$ , scales linearly with the particle diameter. Thus, by designing the

-12-

microfluidic flow chamber dimensions for a particular flow rate of a fluid, a laminar two-phase flow may be used as an effective barrier against particle cross-transport. In the example shown in Figure 6, each of the inlet streams has a width of about 30  $\mu\text{m}$  and the central channel has a length from the inlet ports to the outlet ports of about 3000  $\mu\text{m}$ , the reduction of which will correspondingly reduce the diffusion within the channel 146 for a constant flow rate. Both of the fluids streams 152 and 154 shown are water. The first stream 152 includes a molecular dye (Methylene Blue), which has a diffusion coefficient on the order of about  $1 \times 10^{-5} \text{ cm}^2/\text{sec}$  in water.

Further, as shown by the dashed line in Figure 6a, a portion of the second fluid stream 154 can exit the central channel 146 via the first outlet port 148 while the remainder of the second fluid 154 exits via the second outlet port 150. If the first fluid 152 is a suspension including suspended particles and the second fluid 154 is a clean solvent, for example, the portion of the solvent that exits the first outlet port 148 along with the suspension 152 acts as an additional barrier to cross-contamination of the streams through diffusion. Thus, particles that diffuse into this portion of the solvent stream may still exit the central chamber 146 via the first outlet port 148, as shown in Figure 6.

The steady state flow-based particle barrier can be penetrated, however, by providing an actuator to move a particle 156 across the barrier. A selective activation of an electric, magnetic or optical field, or any combination of these fields, for example, may be used to move the particle 156 from one stream to another stream. Alternatively, a mechanical actuator, such as a valve, pump, gate or applied pressure may be employed to move the particle from one stream to another stream. Although described here for parallel flows, the flows traveling in arbitrary orientations, including opposite directions, are possible.

Figures 6a-6c show a particle 156 being separated from the first inlet stream 152 into the second inlet stream 154 in the embodiment shown in Figure 6. In Figure 6a, a suspension enters the central channel 146 from the first inlet port 142, and a second fluid 154, such as a solvent, enters the central channel 146 from the second inlet channel 144. The suspension 152 and the second fluid 154 flow in a laminar manner through the central channel 146. The suspension stream 152 and a portion of the second fluid stream 154 exit the central channel 146 via the first outlet port 148. The remaining portion of the

-13-

second fluid stream 154 functions as a collection stream and exits the central channel 146 via the second outlet port 150. A particle 156 suspended in the suspension stream 152 is shown entering the central channel 146 from the first inlet port 142, where it is identified as described above. In Figure 6b, the particle 156 is shown being separated from the suspension stream 152 into the second fluid stream 154. The particle 156 may be separated from the suspension 152 via an electrical, magnetic, mechanical or chemical actuator such as described above. In Figure 6c, the particle 156 is shown exiting the central channel 146 via the second outlet port 150 in the second fluid stream 154 for collection.

Figure 7 shows another embodiment of a microfluidic flow chamber 160 in which a particle of interest may be separated from a suspension. The microfluidic flow chamber 160 includes three inlet ports 162, 164 and 166, two outlet ports 168 and 170 and a central channel 172. In this example, a suspension including suspended particles enters from the first inlet port 162. Other fluid streams, such as a pair of solvent or buffer fluid streams enter the central channel 172 from either side of the first inlet port 162. As shown in Figures 7a - 7c, the relative flow rates of each inlet port may be modulated to vary the resulting incoming stream 174 into the central channel 172. In Figure 7a, for example, the relative flow rates of the streams in the second inlet port 164 and the third inlet port 166 are relatively equal and pinch the flow from the first inlet port 162 at a neck and form a narrow stream of the first fluid approximately down the center of the central channel 172. By varying the flow rates of the second and third inlet streams 164 and 166, the width of the first fluid stream 174, i.e., the suspension, can be narrowed down to the width of a single particle. Thus, the inlet sample suspension 174 may be "prefocused" into a narrow, or even single file, particle stream surrounded on either side by a potential collection stream. This allows for a decrease in the lateral distance, i.e., distance perpendicular to the flow direction, a particle must be moved away from the suspension stream to be captured in the collection stream and, thus, an increase in sorting efficiency.

Figure 7b shows the embodiment of Figure 7, wherein the flow rate of the third inlet port 166 is less than the flow rate of the second inlet port 164 and prefocuses the inlet particle stream in the lower half of the central chamber 172. Conversely, Figure 7b shows the embodiment of Figure 7, wherein the flow rate of the third inlet port 166 is greater than the flow rate of the second inlet port 164 and prefocuses the inlet particle

-14-

stream in the upper half of the central chamber 172. The relative flow rates of the three inlets can thus be modulated to control the particle stream within the central channel.

Figure 8 shows yet another embodiment of a microfluidic flow chamber 180 in which a particle of interest may be separated from a suspension. As in Figure 7, the microfluidic flow chamber 180 includes three inlet ports 182, 184 and 186 and a central channel 188. The chamber 180 of Figure 8, however, includes six outlet ports 188, 190, 192, 194, 196 and 198. The number of outlet ports shown in Figure 8 is merely exemplary and may include any number of outlet ports greater than or equal to two. In this example, the plurality of outlet ports may be used to sort a plurality of particles into various outlet ports. Different types of particles, for example, may be sorted into different outlet ports. Alternatively, the plurality of outlet ports may be used to individually sort the same type of particles into different outlet ports. In yet another embodiment, the side flows may be modulated as described above to dispense particles, chemicals and/or fluids (e.g., reagents) into multiple outlet ports for use in various downstream applications or networks.

Alternatively, the incoming streams may be prefocused prior to entry into the microfluidic flow chamber, or the side inlet ports may be arranged to enter the central channel downstream of the first inlet port.

Figure 9 shows an embodiment of a microfluidic flow chamber 200 in which a particle of interest may be separated from a suspension via a mechanical actuator. As shown in Figure 9, the central channel 202 includes a side channel 204 through which incoming fluid flow is controlled by a valve 206. After a particle is detected, the valve may be opened to vary the fluid flow within the central channel 202 and divert the suspension along with the particle away from the first outlet port 208 into the second outlet port 210. Alternatively, the valve 206 may be closed or the flow through the valve may be merely adjusted to divert the particle into the desired outlet port. Similarly, the valve 206 may be positioned on the opposite side of the central chamber 202 and may obtain a similar result by providing or modulating the flow in the opposite direction.

Figures 9a – 9c show yet another embodiment of a microfluidic flow chamber 220 in which a particle of interest may be separated from a suspension via a mechanical actuator. As shown in Figure 9a, the particle 222 enters the central channel 224 in the suspension via the first inlet port 226. In Figure 9b, the valve 228 activates after the

particle is identified as described above and redirects the particle 222 into the second outlet port 230. Then, in Figure 9c, after the particle 222 has exited the central channel 224, the valve 228 retracts and the fluid stream flows return to their steady state condition.

5           Figures 9d – 9f show an exemplary microfluidic flow chamber 240 in which a particle of interest may be separated from a suspension via a chemical actuator. As shown in Figures 9d – 9f, the microfluidic flow chamber 240 includes a chemical actuator material 242, such as a hydrogel, that swells or shrinks in reaction to an attribute associated with a particular particle of interest (e.g., pH). Hydrogels, such as these are  
10       known in the art. Beebe, David J. et al, “Functional Hydrogel Structures for Autonomous Flow Control Inside Microfluidic Channels, *Nature*, vol. 404, pp. 588-90, (April 6, 2000), for example, discloses hydrogel actuators that may be used in the present embodiment.

          Figures 9d – 9f show a chemically actuated valve 244 including the chemical actuator material 242. In Figure 9d, for example, the chemical actuator is in its normal  
15       condition in which the valve 244 is open and the suspension flows through the first outlet port 246. Figure 9e shows the chemical actuator in its active state in which the chemical actuator material 242 is swollen in response to a detected attribute, effectively shutting off the first outlet port 246 and the suspension flows through the second outlet port 252 and allowing the particle 250 of interest to be collected. Although Figure 9e shows the  
20       chemically actuated valve 244 completely closing off the first outlet port, the swelling of the chemically actuated material 242 may also merely create a barrier to particular-sized particles while allowing the remainder of the suspension to pass into the first outlet port 246. Where the individual valve members are angled toward the second outlet port 252, the blocked particles 250 may be conveyed to the second outlet port 252 for collection.  
25       Figure 9f further shows the chemically actuated valve 244 returned to its open condition after the detected particle 250 has passed into the second outlet port 252.

          Alternatively microfluidic flow devices may employ laminar flows and specific microgeometries for non-actuated separation of colloidal and/or cellular particles in fluid suspensions. The geometry of these devices has been designed to act similarly to a filter  
30       without the use of membranes or sieves which are highly susceptible to clogging and fouling. Such devices will also be capable of replacing the centrifugation step common to many biological processes upon a chip surface. With a microscale alternative to



centrifugation available, a host of multi-step biological processes such as bead-based assays and cell counting using dying techniques will be able to be performed within microfluidic devices.

As demonstrated in Figures 10 – 12, specific channel geometries may be created to take advantage of the laminar nature of fluids flowing in microchannels. In each of these designs, the particle suspension enters the central channel 260 through a first inlet port 262. A second fluid stream, such as a solvent stream, enters the channel 260 through a second inlet port 264, which meets the first inlet port 262 at any angle. Because of the laminar nature of microfluidic flows, these streams will generally not mix convectively. The central channel 260 further includes microscale obstacles 265. Molecular debris small enough to fit through the openings formed by the microscale obstacles 265 will be carried down the first outlet port 266. Due to the presence of microscale obstacles, however, any particles larger than the separation of the obstacles will be shuffled toward the second outlet port 268 and exit the central channel 260 with a portion of the second fluid stream. The designs shown here do not depend upon relative channel size, instead the presence of the microscale obstacles at or near the confluence of the two (or more) inlet streams alter the direction of flow for any particulate matter in the suspension inlet stream(s).

Figure 13 further shows a configuration for sorting particles in the suspension by size and produces a size fractionation effect by designing the size of the gaps 274 between the guides 276 to increase away from the first inlet port 262, by which the suspension is introduced into the central channel 270. By gradually increasing the widths of the gaps 274 moving away from the first inlet port 262, particles of increasing size flow into the guides 276 and may be collected individually.

Figure 14 shows yet another embodiment of a non-actuated separation of motile particles within a suspension between laminar flows. In this embodiment, motile particles 280 entering in the suspension flow 282 move within the suspension flow and can pass from the suspension flow 282 into the second fluid stream 284 without the need of an actuator to separate the particles 280 from the suspension flow 282. In this manner, the motile particles 280 may enter the second fluid stream 284 and exit the central channel 286 through the second outlet port 290 instead of the first inlet port 288. For example, in a suspension 282 containing sperm, the active sperm may move on their own into the

-17-

second fluid stream 284 for collection, while inactive sperm are carried out of the central channel 286 with the suspension 282 via the first outlet port 288.

Non-actuated separation of colloidal and/or cellular particles from a suspension in a microfluidic flow device presents a very simple approach to microfluidic separations or enrichments of colloidal and/or cellular particles because it relies upon the condition native to fluids flowing on the microscale, regardless of flow rate or channel morphology: laminar flows. Furthermore, the selection of materials for the construction of these devices is irrelevant, thus they may be incorporated into microfluidic devices constructed on any substrate.

Figure 15 shows another example of a microfluidic flow chamber in which a series of discrete sample suspensions 300 are combined into a single laminar flow. In this example, a plurality of discrete samples 300 form the single sample flow. The sample flow further preferably includes buffers 302 between each discrete sample 300 to prevent cross-contamination between samples 300. In this manner, a single microfluidic flow chamber 304 can separate particles from a series of samples to increase throughput. The series of discrete sample suspensions may, for example, be created using a microfluidic dispenser as shown and described above with reference to Figure 8 in which individual samples are directed into a plurality of outlet ports and combined downstream into a series of discrete sample streams.

Figure 16 shows a cartridge 310 that may be plugged into, or otherwise connected to, a system for separating one or more colloidal or cellular particles from a suspension. The cartridge 310 may be reusable or disposable. The cartridge may include a sample reservoir 312, or other inlet mechanism, for receiving a fluid suspension. The sample reservoir 312 is connected to a central channel 314 via a first inlet port 316. The cartridge further includes a waste receptacle 318, or other outlet mechanism, connected to the central channel 314 via a first outlet port 320 for receiving the suspension after it has passed through the central channel 314 for the removal of one or more particles of interest. A collection receptacle 322 is also connected to the central channel 314 via a second outlet port 324 for receiving the particles collected from the suspension. The collection receptacle 322 may include a reservoir or other means for holding the collected particles or may include a channel or other means for providing the collected particles to downstream networks for further processing.

The cartridge 310 may also include a second inlet reservoir 326 for receiving a second fluid, may receive the second fluid from an external source in the system, or may not utilize a second fluid at all, such as described with reference to Figure 5. If used, the second fluid may include a fluid such as a buffer or a solvent (e.g., water, a saline suspension and the like) or a reagent (e.g., antibody tagged particles, fluorescent tags, lysing agents, anticoagulants and the like), or any combination thereof. Indeed, the fluid requirements may be system-specific and may be matched to the intended application and mode of use. The second inlet reservoir 326 or receptacle for receiving a second fluid, if used, may be connected to the central channel 314 via a second inlet port 328.

The reservoirs or receptacles may include any interface for transferring a fluid known in the art. For example, the reservoir may be adapted to receive fluids from a syringe, either with or without a needle, from a tube, from a pump, directly from a human or animal, such as through a finger stick, or from any specially designed or standard fluid transfer coupling.

The microfluidic flow chambers described herein may be manufactured by a variety of common microelectronics processing techniques. A pattern of a shadow mask may be transferred to a positive or negative photoresist film spun upon a silicon wafer, a glass slide, or some other substrate, for example. This pattern may be sealed and used directly as the microfluidic network, replicated in another material, or further processed.

The substrate may be further processed through subsequent wet etching, dry etching, molecular epitaxy, physical deposition of materials, chemical deposition of materials, and the like, or any combination of these or similar techniques. The final network may be used directly or reproduced through the use of a replication technique designed to produce a replica upon the master, such as by the pouring and curing, imprinting in or deposition of elastomers, polymers and the like. A pump or other means for introducing and controlling fluid flow within the fluidic network as well as a means for connecting the pump or pressure differential means may also be provided. The network can further be sealed, such as with a cover slip, glass slide, silicon wafer, polymer films or a similar substrate.

In one specific, nonlimiting example, a pattern on a shadow mask was exposed to ultraviolet light and transferred to a negative photoresist film spun upon a silicon wafer to a depth of approximately 5  $\mu\text{m}$ . A two-part mixture of poly(dimethyl siloxane) (PDMS),

-19-

which is commercially available from Dow Corning under the trade name of Sylgard 184, was poured and cured upon the silicon master to produce a flexible, biocompatible optically transparent replica. In addition to the PDMS channel network a flow apparatus comprising a syringe pump such as a kdScientific, model 200 syringe pump and a polymethyl methacrylate (PMMA) flow introduction base. The PDMS channel network was placed upon the PMMA base, and holes were punched through the PDMS to provide access for the microchannels to the ports in the base. The network was further sealed with a cover slip. Because the PDMS forms a tight seal with both PMMA and glass, no additional bonding or clamping was required. The syringe pump was further fitted with 3 cm<sup>3</sup> plastic syringes (such as available from Becton-Dickson) joined to the base.

One embodiment of an optical trap and digital microscopy that may be used with the microfluidic flow devices described herein may incorporate a piezoelectric mirror (such as available from Physik Instrumente, model S-315) to simultaneously trap several particles by rapidly scanning a single laser beam (such as available from Spectra Physics, 532 nm, typically operated at 200mW) among a number of positions to create a time-averaged extended trapping pattern. A Neofluar, 100X, oil immersion high numerical aperture objective (N.A. = 1.30) can be used to focus the beam and create the optical trap. CCD images can be captured by a data acquisition board and processed by LabView (National Instruments) routines that may be customized to distinguish various visual particle or cell features for specific applications. Optical traps and digital microscopy are described in further detail, for example, in Mio, C.; Gong, T.; Terry, A.; Marr, D.W.M., Design of a Scanning Laser Optical Trap for Multiparticle Manipulation, Rev. Sci. Instrum. 2000, 71, 2196-2200.

While the invention has been particularly shown and described with reference to particular embodiment(s) thereof, it will be understood by those skilled in the art that various other changes in the form and details may be made without departing from the spirit and scope of the invention. One skilled in the art of microfluidic flows, for example, would recognize that downstream or upstream analogues of mechanisms described herein may be substituted for the particular exemplary structures disclosed herein.

-20-

We claim:

1. A microfluidic flow device for separating a particle within a suspension flow, the microfluidic flow device comprising:

5 a microfluidic channel comprising an inlet port for receiving the suspension flow under laminar conditions, a first outlet port and a second outlet port; and

an interface for translating the particle within said channel, wherein said first outlet port is adapted to receive a first portion of the suspension exiting said channel and said second outlet port is adapted to receive the particle in a second portion of the suspension exiting the channel.

10

2. The microfluidic flow device of claim 1, wherein said interface comprises an optically transparent portion of said channel.

15 3. The microfluidic flow device of claim 1, wherein said interface comprises two or more electrodes to provide an electric field in the central channel.

4. The microfluidic flow device of claim 3, wherein said interface further comprises an electrical interconnect for receiving electrical energy from the system and providing the electrical energy to said two or more electrodes.

20

5. The microfluidic flow device of claim 1, wherein said interface further comprises a magnet for providing a magnetic field extending into said channel.

25 6. The microfluidic flow device of claim 1, wherein said channel is adapted such that an electric field may extend into said channel for translating the particle within the suspension flow.

7. The microfluidic flow device of claim 6, wherein said field comprises at least one or more of an optical trap, an electrical field and a magnetic field.

30

-21-

8. The microfluidic flow device of claim 1, where in said interface comprises a channel geometry for sorting the particle within the channel.

9. The microfluidic flow device of claim 1, wherein said laminar conditions  
5 comprise a Reynolds number of less than about 1000.

10. The microfluidic flow device of claim 1, wherein said channel comprises a cartridge.

10 11. The microfluidic flow device of claim 1, wherein said channel comprises a disposable cartridge.

12. The microfluidic flow device of claim 1 further comprising a pressure differential generator for providing fluid flow in said channel.

15 13. The microfluidic flow device of claim 12, wherein said pressure differential generator comprises one or more of the group comprising: a pump, a capillary force generator, a gravity feed generator, an electro-osmosis system, a syringes, a valve, a suction generator, and a vacuum generator.

20 14. A microfluidic flow device for separating a particle from a suspension flow into a second fluid flow, the microfluidic flow device comprising:  
a microfluidic channel comprising a first inlet port for receiving the suspension flow, a second inlet port for receiving the second fluid flow, a first outlet port and a  
25 second outlet port, wherein said channel is adapted to receive the suspension flow and the second fluid flow under laminar conditions; and  
an interface for translating the particle from the suspension flow to the second fluid flow, wherein said first outlet port is adapted to receive at least a portion of the suspension flow exiting said channel and said second outlet port is adapted to receive the  
30 particle in at least a portion of the second fluid flow exiting said channel.

-22-

15. The microfluidic flow device of claim 14, wherein said interface comprises an optically transparent portion of said channel.

16. The microfluidic flow device of claim 14, wherein said interface comprises  
5 two or more electrodes to provide an electric field in the central channel.

17. The microfluidic flow device of claim 16, wherein said interface further  
comprises an electrical interconnect for receiving electrical energy from the system and  
providing the electrical energy to said two or more electrodes.  
10

18. The microfluidic flow device of claim 14, wherein said interface further  
comprises a magnet for providing a magnetic field extending into said channel.

19. The microfluidic flow device of claim 14, wherein said channel is adapted  
15 such that an electric field may extend into said channel for translating the particle within  
the suspension flow.

20. The microfluidic flow device of claim 19, wherein said field comprises at  
least one or more of an optical trap, an electrical field and a magnetic field.  
20

21. The microfluidic flow device of claim 14, where in said interface comprises a  
channel geometry for sorting the particle within the channel.

22. The microfluidic flow device of claim 14, wherein said laminar conditions  
25 comprise a Reynolds number of less than about 1000.

23. The microfluidic flow device of claim 14, further comprising a third inlet  
port.

24. The microfluidic flow device of claim 23, wherein said second and third inlet  
30 ports are adapted for providing a modulated flow rate

-23-

25. The microfluidic flow device of claim 14, wherein said channel is adapted to receive the suspension flow and the second fluid flow in parallel flows in the same direction.

5

26. The microfluidic flow device of claim 14, wherein said channel is adapted to receive the suspension flow and the second fluid flow in parallel flows in the opposite direction.

10

27. The microfluidic flow device of claim 14, wherein said channel comprises a cartridge.

28. The microfluidic flow device of claim 27, wherein said channel comprises a disposable cartridge.

15

29. The microfluidic flow device of claim 14 further comprising a pressure differential generator for providing fluid flow in said channel.

30. The microfluidic flow device of claim 29, wherein said pressure differential generator comprises one or more of the group comprising: a pump, a capillary force generator, a gravity feed generator, an electro-osmosis system, a syringes, a valve, a suction generator, and a vacuum generator.

20

31. The microfluidic flow device of claim 14, further comprising a third inlet port for receiving a third fluid flow.

25

32. The microfluidic flow device of claim 31, wherein said second inlet port enters said channel at a first angle to said first inlet port and said third inlet port enters said channel at a second angle to said first inlet port.

30



-24-

33. The microfluidic flow device of claim 32, wherein said second inlet port and said third inlet port provide a means for orienting the suspension flow in the channel.

34. The microfluidic flow device of claim 33, wherein said means for orienting the suspension flow is adapted to orient the suspension flow linearly.

5

35. The microfluidic flow device of claim 33, where in said means for orienting the suspension flow is adapted to modulate the position of the suspension flow within said channel.

10

36. A method of separating a particle within a suspension comprising:  
receiving a suspension flow in a microfluidic channel, the suspension flowing under laminar conditions;  
translating a particle within the suspension flow;  
exiting a first portion of the suspension flow through a first outlet port, and  
15 exiting the particle along with a second portion of the suspension flow through a second outlet port.

20

37. The method of claim 36, wherein the translating of the particle includes the application of a field.

38. The method of claim 37, wherein the translating of the particle includes the application of one or more of the group comprising: an electric field, an optical field and a magnetic field.

25

39. The method of claim 36, wherein the translating of the particle comprises using a channel geometry for sorting the particle within the channel.

40. The method of claim 36, wherein the laminar conditions comprise a Reynolds number of less than about 1000.

30

-25-

41. The method of claim 36 further providing a pressure differential for providing fluid flow in the channel.

42. The method of claim 41, wherein the pressure differential is provided by one  
5 or more of the group comprising: a pump, a capillary force generator, a gravity feed generator, an electro-osmosis system, a syringes, a valve, a suction generator, and a vacuum generator.

43. A method of separating a particle from a suspension flow comprising:  
10 receiving a suspension flow in a microfluidic channel, the suspension flowing under laminar conditions;  
receiving a second fluid flow in the channel, the suspension and the second fluid flowing under laminar conditions in the channel;  
separating a particle from the suspension flow into the second fluid flow;  
15 exiting at least a portion of the suspension flow through a first outlet port; and  
exiting the particle in at least a portion of the second fluid flow through a second outlet port.

44. The method of claim 43, wherein the translating of the particle includes the  
20 application of a field.

45. The method of claim 44, wherein the translating of the particle includes the application of one or more of the group comprising: an electric field, an optical field and a magnetic field.  
25

46. The method of claim 43, wherein the translating of the particle comprises using a channel geometry for sorting the particle within the channel.

47. The method of claim 43, wherein the laminar conditions comprise a Reynolds  
30 number of less than about 1000.

-26-

48. The method of claim 43 further providing a pressure differential for providing fluid flow in the channel.

49. The method of claim 48, wherein the pressure differential is provided by one  
5 or more of the group comprising: a pump, a capillary force generator, a gravity feed generator, an electro-osmosis system, a syringes, a valve, a suction generator, and a vacuum generator.

50. A cartridge for use in system to separate a particle from a suspension flow,  
10 the cartridge comprising:  
a microfluidic channel comprising an inlet port for receiving a suspension flow under laminar conditions, a first outlet port and a second outlet port; and  
an interconnect for connecting the cartridge to the system,  
wherein said microfluidic channel is adapted to receive the suspension flow and  
15 provide an environment for translating the particle within the suspension flow, said first outlet port is adapted to receive a first portion of the suspension flow, and said second outlet port is adapted to receive the particle in a second portion of the suspension flow.

51. The cartridge of claim 50 further comprising an interface for translating the  
20 particle within said channel.

52. The cartridge of claim 51, wherein said interface comprises an optically transparent portion.

25 53. The cartridge of claim 51, wherein said interface comprises two or more electrodes to provide an electric field in the central channel.

54. The cartridge of claim 51, wherein said interface further comprises an electrical interconnect for receiving electrical energy from the system and providing the  
30 electrical energy to said two or more electrodes.

-27-

55. The cartridge of claim 51, wherein said interface further comprises an magnet for providing a magnetic field extending into said channel.

56. The cartridge of claim 50, wherein said channel is adapted such that a field  
5 may extend into said channel for translating the particle within the suspension flow.

57. The cartridge of claim 56, wherein said field comprises at least one or more of an optical trap, an electrical field and a magnetic field.

10 58. A microfluidic flow separator comprising:  
a channel means for receiving a suspension flow under laminar conditions;  
a translating means for translating a particle within the suspension flow; and  
a first output means for exiting a first portion of the suspension flow; and  
a second output means for exiting the particle in a second portion of the  
15 suspension flow.

59. A cartridge for use in system to separate a particle from a suspension flow into a second fluid flow, the cartridge comprising:

a microfluidic channel comprising a first inlet port for receiving the suspension  
20 flow, a second inlet port for receiving the second fluid flow, a first outlet port and a second outlet port, wherein said channel is adapted to receive the suspension flow and the second fluid flow under laminar conditions,  
an interconnect for connecting the cartridge to the system,  
wherein said microfluidic channel is adapted to provide an environment for  
25 translating the particle from the suspension flow to the second fluid flow, said first outlet port is adapted to receive at least a portion of the suspension flow, and said second outlet port is adapted to receive the particle in at least a portion of the second fluid flow.

60. The cartridge of claim 59 further comprising an interface for translating the  
30 particle within said channel.

-28-

61. The cartridge of claim 60, wherein said interface comprises an optically transparent portion.

5 62. The cartridge of claim 60, wherein said interface comprises two or more electrodes to provide an electric field in the central channel.

63. The cartridge of claim 60, wherein said interface further comprises an electrical interconnect for receiving electrical energy from the system and providing the electrical energy to said two or more electrodes.

10

64. The cartridge of claim 60, wherein said interface further comprises an magnet for providing a magnetic field extending into said channel.

65. The cartridge of claim 59, wherein said channel is adapted such that a field  
15 may extend into said channel for translating the particle within the suspension flow.

66. The cartridge of claim 65, wherein said field comprises at least one or more of an optical trap, an electrical field and a magnetic field.

20 67. A microfluidic flow separator comprising:  
a channel means for receiving a suspension flow and a second fluid flow, the suspension flow and the second fluid flow under laminar conditions;  
a separator means for separating a particle from the suspension flow into the second fluid flow; and  
25 a first output means for exiting at least a portion of the suspension flow; and  
a second output means for exiting the particle in at least a portion of the second fluid flow.

68. A microfluidic chemical dispenser for dispensing a first fluid flow into a  
30 plurality of receptacles comprising:  
a first inlet port for receiving the first fluid flow;

-29-

a second inlet port for receiving a second fluid flow at a first angle to the first fluid flow;

a third inlet port for receiving a third fluid flow at a second angle to the first fluid flow;

5 a central channel for receiving the first fluid flow, the second fluid flow and the third fluid flow under laminar conditions;

a plurality of outlet ports for receiving fluid flow from the central channel;

a modulator means for modulating the relative flow rates of the second fluid stream and the third stream to dispense the first fluid flow into said plurality of outlet  
10 ports.

69. The microfluidic dispenser of claim 68, wherein the first fluid flow comprises a buffer fluid flow.

15 70. The microfluidic dispenser of claim 68, wherein the first fluid flow comprises a solvent fluid flow.

71. A system for separating a particle from a solution in a microfluidic flow device, the system comprising:

20 a microfluidic channel comprising an input port, a first output port and a second output port, said channel being adapted to receive the suspension via the input port under laminar conditions;

a detector for monitoring said channel and providing an output;

25 an information processor for receiving said output and determining if the particle is present in said channel; and

an actuator for translating the particle within said channel, wherein said information processor triggers said actuator if the particle is detected.

1/13

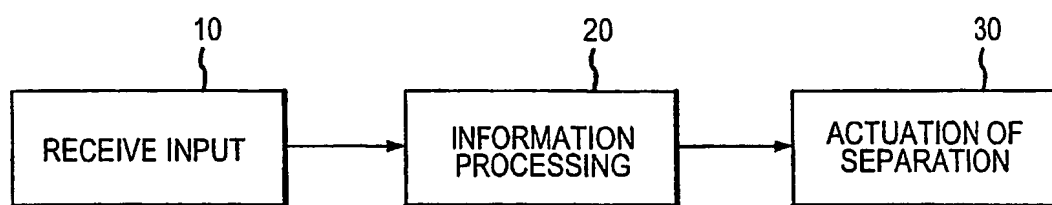


FIG. 1

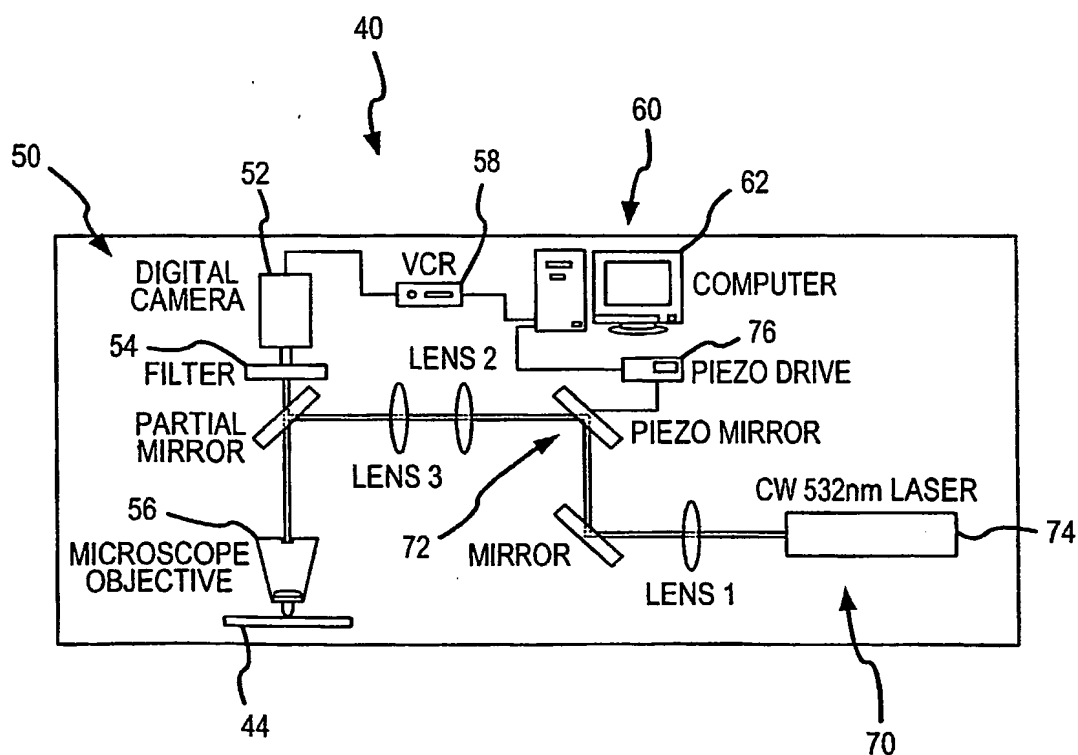


FIG. 2

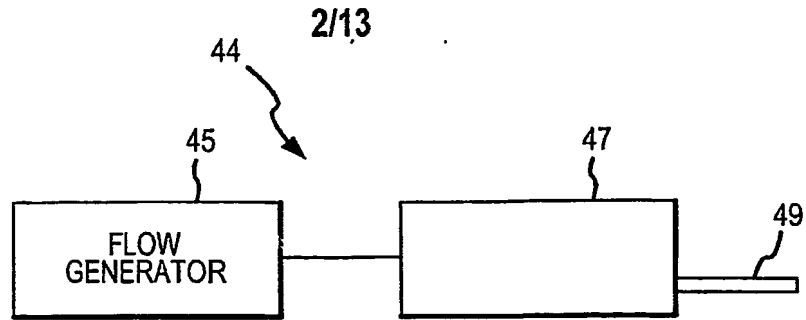


FIG. 2A

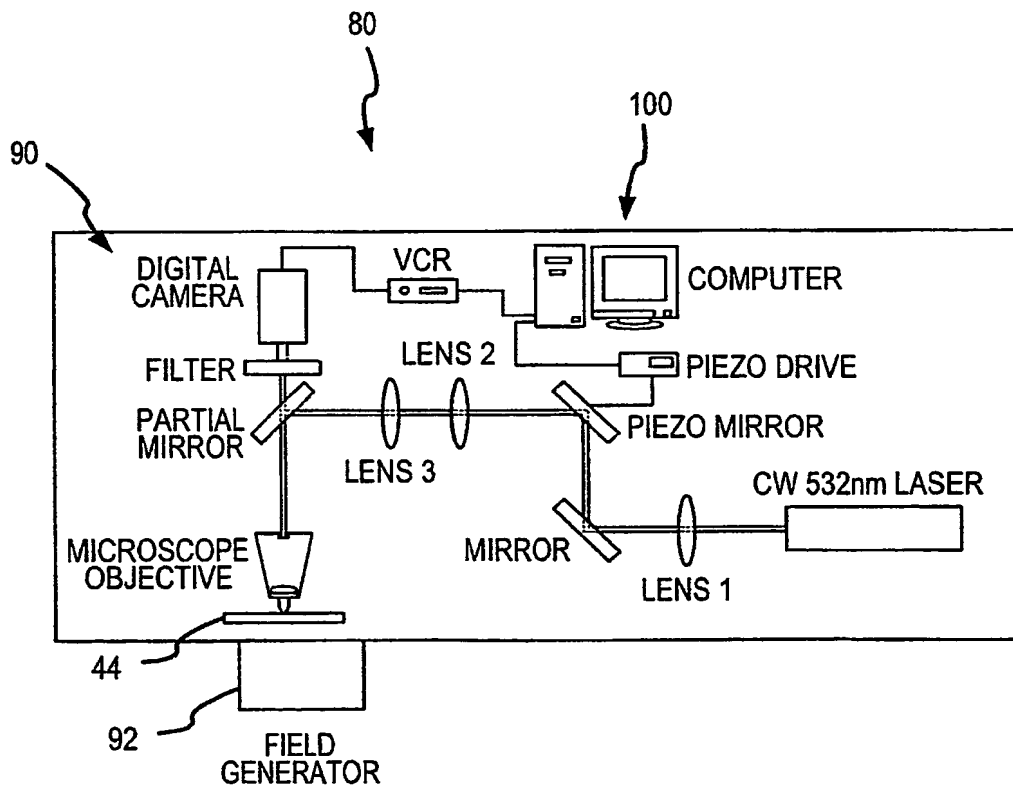


FIG. 3



3/13

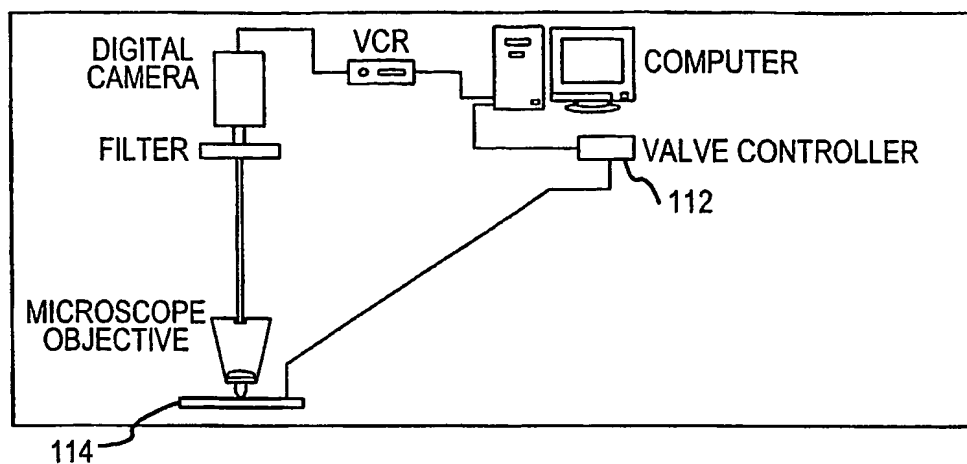


FIG.4

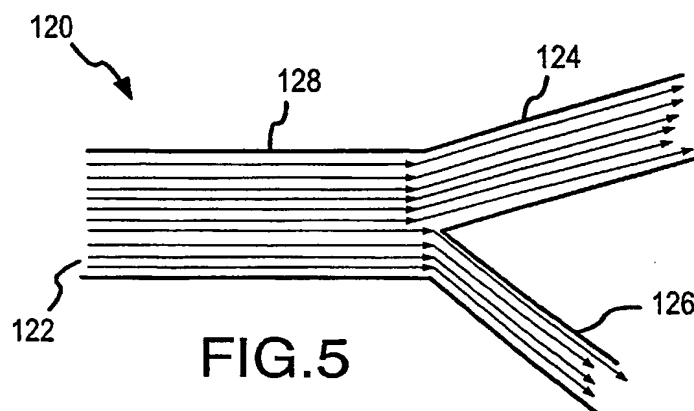


FIG.5

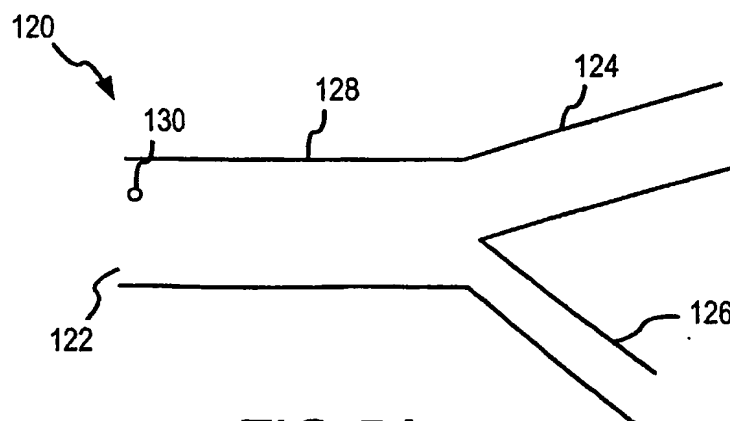


FIG.5A

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4/13

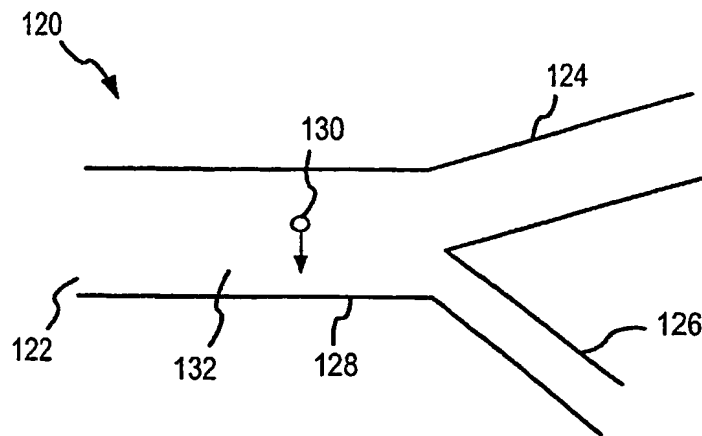


FIG. 5B

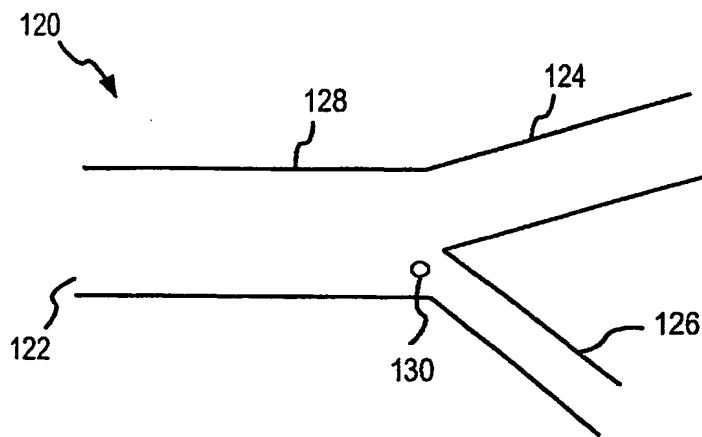


FIG. 5C

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5/13

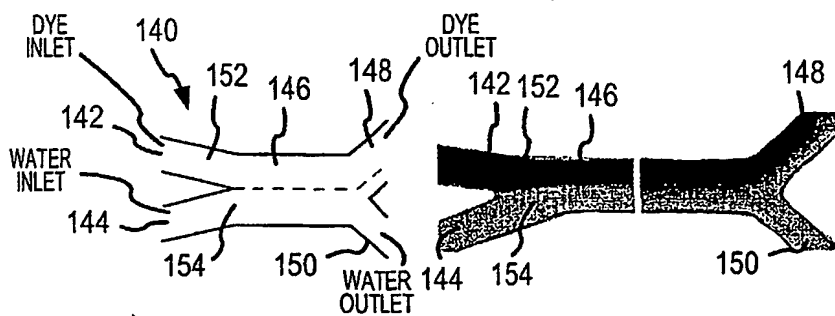


FIG. 6

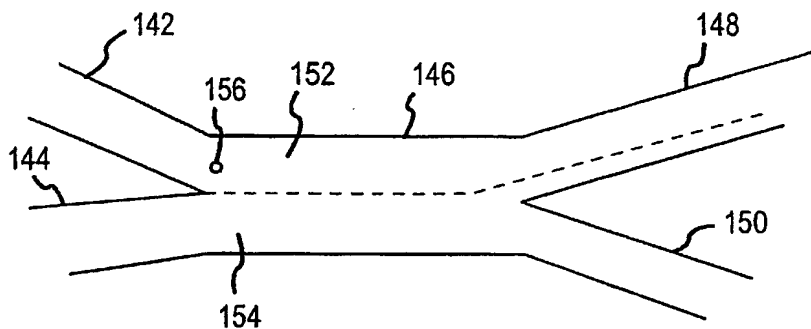


FIG. 6A

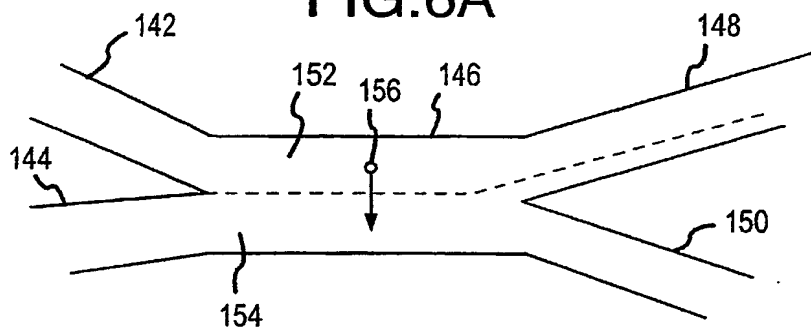


FIG. 6B

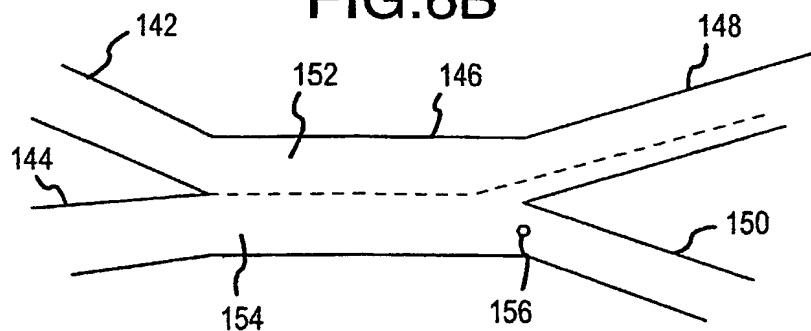


FIG. 6C

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6/13

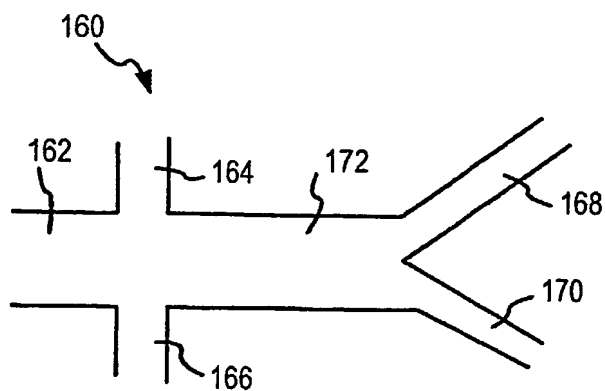


FIG. 7

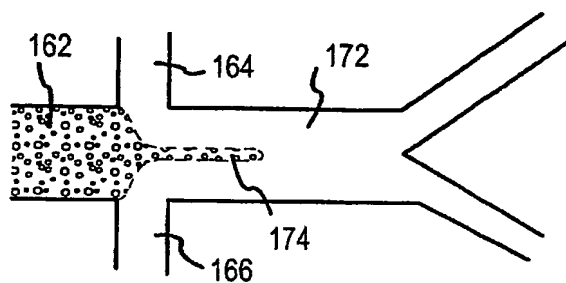


FIG. 7A

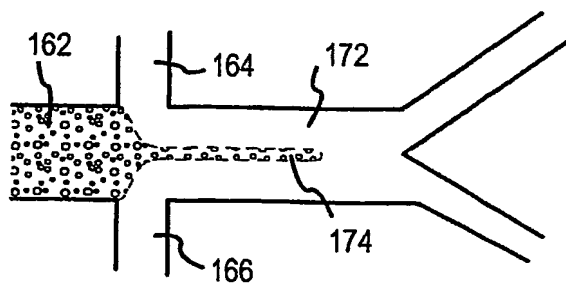


FIG. 7B

7/13

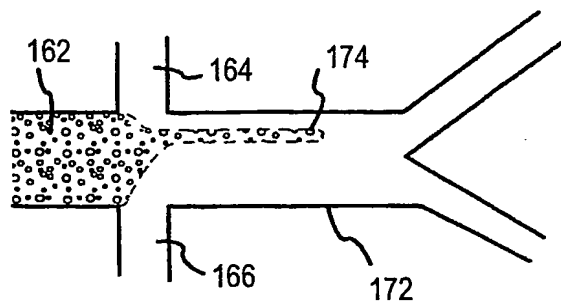


FIG. 7C

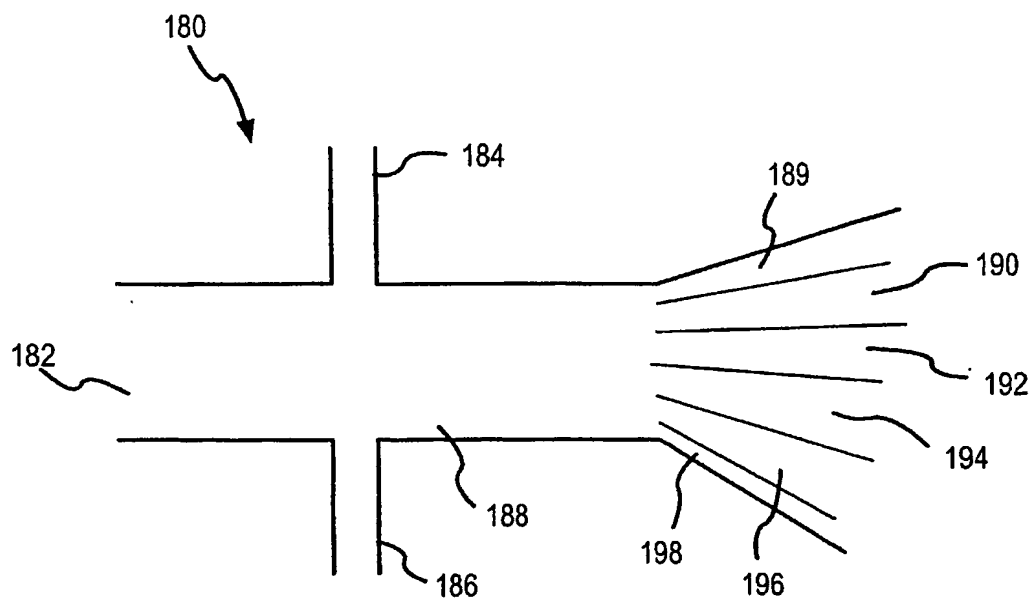


FIG. 8

SUBSTITUTE SHEET (RULE 26)

8/13

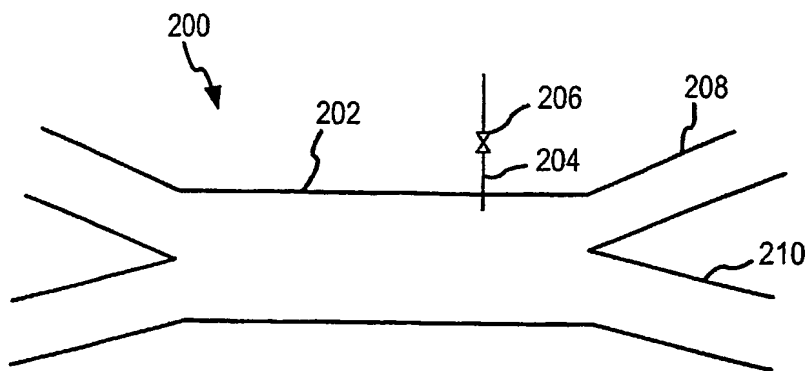


FIG. 9

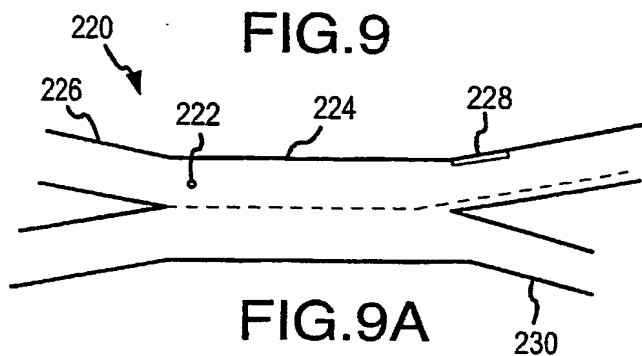


FIG. 9A

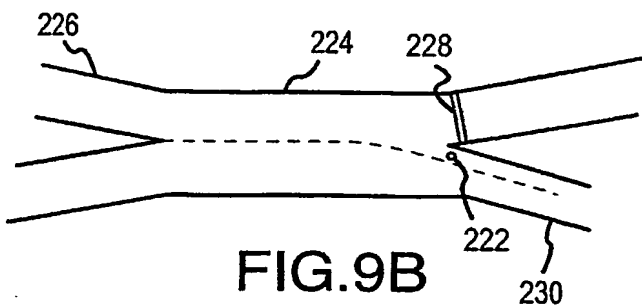


FIG. 9B

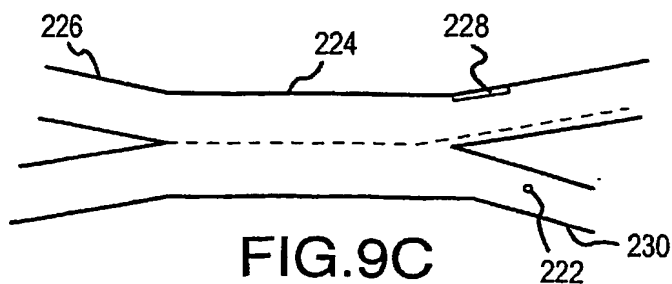


FIG. 9C

SUBSTITUTE SHEET (RULE 26)

9/13

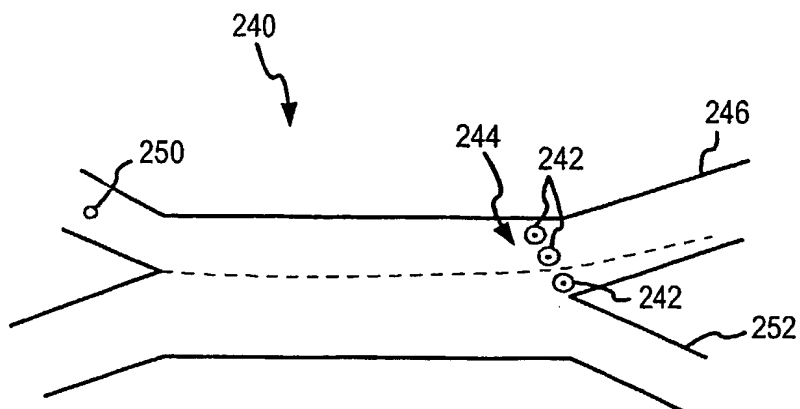


FIG. 9D

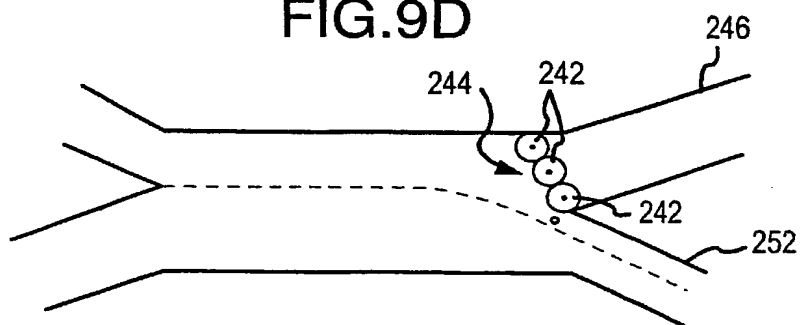


FIG. 9E

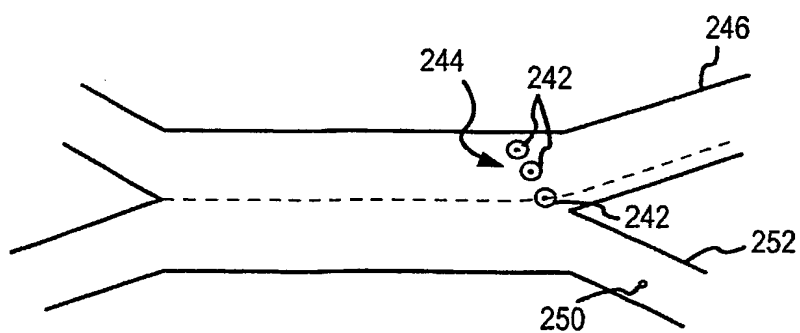


FIG. 9F

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10/13

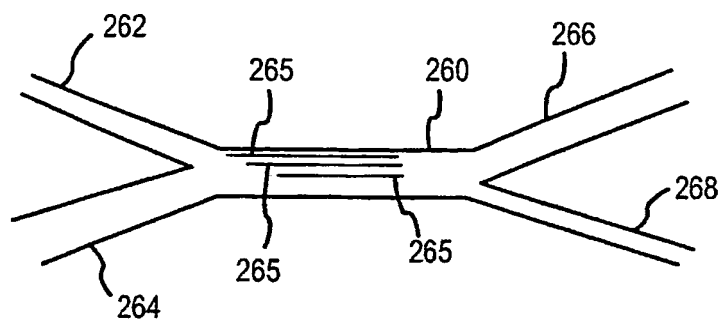


FIG. 10

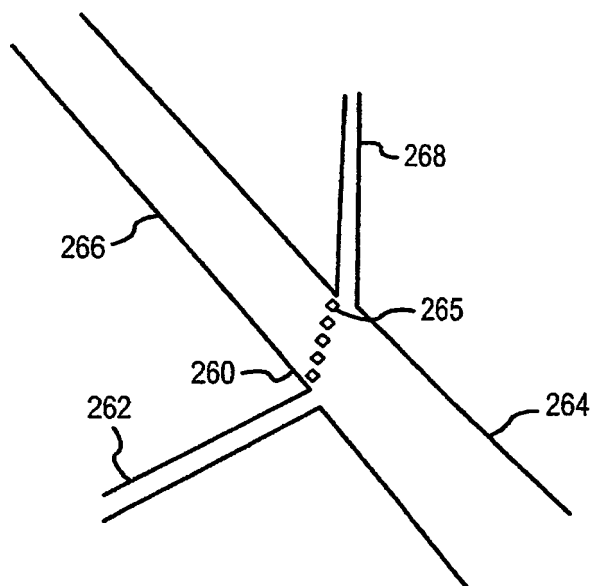


FIG. 11

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11/13

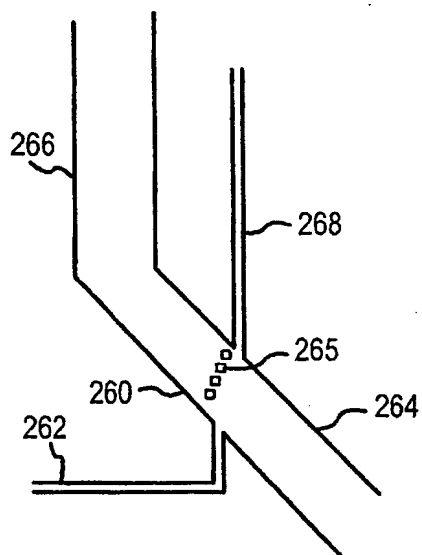


FIG. 12

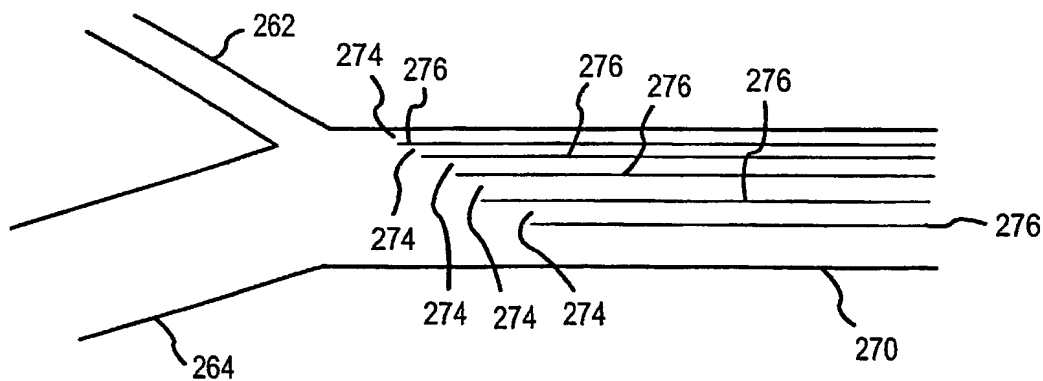


FIG. 13

SUBSTITUTE SHEET (RULE 26)

12/13

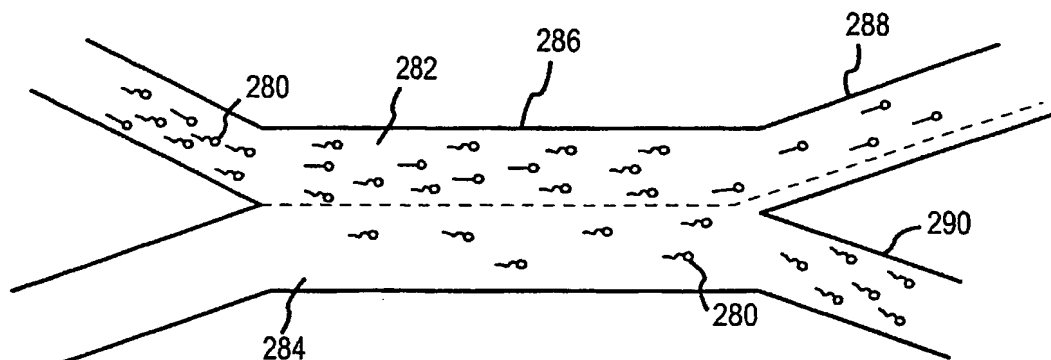


FIG. 14

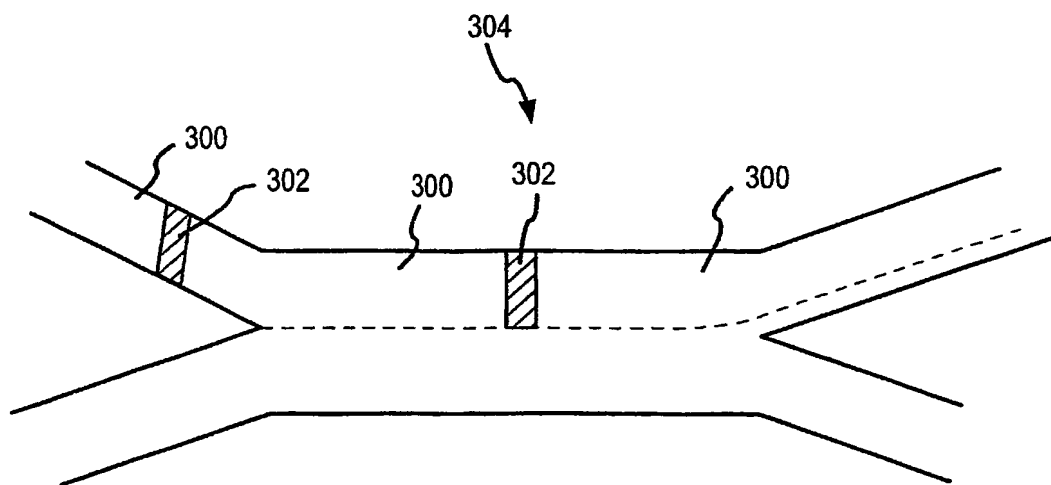


FIG. 15

SUBSTITUTE SHEET (RULE 26)

13/13

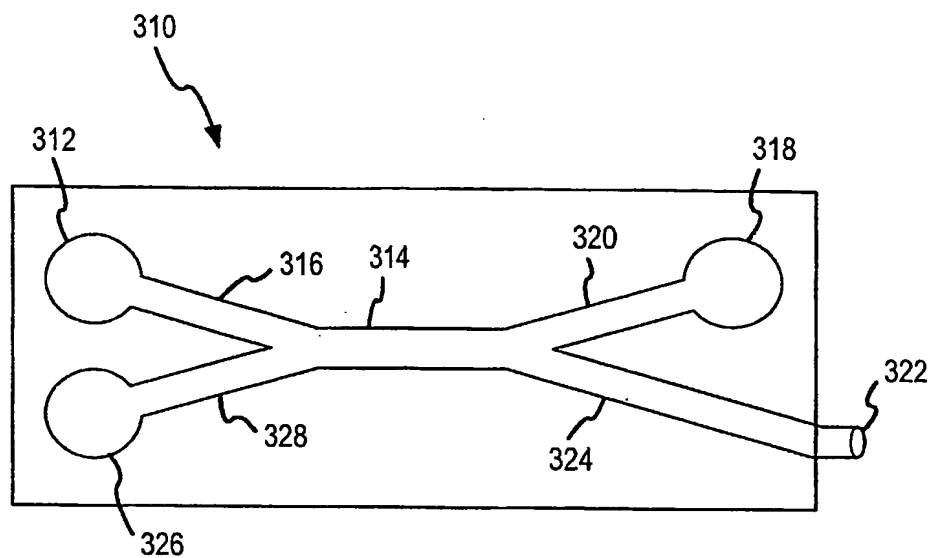


FIG.16

SUBSTITUTE SHEET (RULE 26)

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/03480

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : BO1D 37/00,37/02,57/02; BO1F 5/06;BO3C 1/00,1/035;GO1N 1/00,15/14, 33/53,33/543,33/553  
US CL : 204/451,453,601,603;210/222,243,695,767;366/336;422/50,101,186.01,186.03,186.1;436/180,514,518,526  
According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 204/451,453,601,603;210/222,243,695,767;366/336;422/50,101,186.01,186.03,186.1;436/180,514,518,526

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98/10267 A1 (TECHNICAL UNIVERSITY OF DENMARK) 12 March 1998 (12.03.1998), page 3, line 18 to line 35, page 4, lines 10-32, page 5, line 25 to page 7, line 15, and page 8, line 4 to page 9, line 33.	1-70



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	"&" document member of the same patent family

Date of the actual completion of the international search

15 May 2003 (15.05.2003)

Date of mailing of the international search report

16 JUN 2003

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, Virginia 22313-1450

Facsimile No. (703)305-3230

Authorized officer

David A. Reifsnnyder

Telephone No. 1-703-308-3601

Form PCT/ISA/210 (second sheet) (July 1998)

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/03480

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐  
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

PCT/US03/03480

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-49, 58 and 67-70 drawn to a microfluidic flow channel and a method of using the microfluidic flow channel to separate a particle within a suspension in that flow channel.

Group II, claims 50-57 and 59-66, drawn to a cartridge for use in a system for separating a particle from a suspension flow into a second flow.

Group III, claim 71, drawn to a control system for separating a particle for a solution in a microfluidic flow channel.

The inventions listed as Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Each of Groups I-III have a Special Technical Feature that is not in another Group.

Group I is a device and process of using that device, the device being a Microfluidic Flow Channel, the Special Technical Feature being an Interface for Translating the Particle.

Group II is a Cartridge for use in a System, the Cartridge including a Microfluidic Flow Channel, the Special Technical Feature being an Interconnect for connecting the Cartridge to the System.

Group III is a Control System including a Microfluidic Flow Channel, the Control System's Special Technical Feature being a Detector, and a Information Processor.

The Special Technical Feature that is shared by Groups I-III is the Channel adapted to receive a Suspension Flow and provide an environment for translating a Particle within the Suspension, the Channel including a First Outlet Port to receive a First Portion of the Suspension exiting said Channel and a Second Outlet Port adapted to receive the Particle in a Second Portion of the Suspension exiting said Channel. The Special Technical Feature is shown in WO 98/10267; therefore the Special Technical Feature does not define a contribution over the prior art of record.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows:

The Species of Group I are as follows:

Species	Corresponding Claim Numbers
1-channel and process-channel having one inlet port and two outlet ports.	1-13, 36-42 and 58
2-channel and process-channel having two inlet ports and two outlet ports.	14-35, 43-49 and 67
3 channel having three inlet ports and multiple outlet ports.	68-70

The Species of Group II are as follows:

Species	Corresponding Claim Numbers
1-cartridge with chanel having one inlet port and two outlet ports.	50-57
2-cartridge with channel having two inlet ports and two outlet ports.	59-66

Form PCT/ISA/210 (second sheet) (July 1998)

# INTERNATIONAL SEARCH REPORT

PCT/US03/03480

The following claim(s) are generic: none.

The species listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: Because as shown in the above tables each Species has a Special Technical Feature that is not in another Species.

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